REMARKS

Claims 1-20 were pending is this application. Applicant has hereinabove canceled claims 1-20, without prejudice. Upon entry of this Amendment new claims 21-46 will be pending. Applicant maintains that the entry of new claims 21-46 does not involve new matter. Support for new claims 21-32 may be found at, *inter alia*, pages 3 and 8-11 of the Specification. Support for new claims 33-46 may be found at, *inter alia*, pages 8-17 of the Specification.

Non-statutory Double Patenting

The Examiner has rejected claims 1-20 for obviousness-type double patenting as being unpatentable over claims 1-10 of U.S. Patent No. 6,346,297. Applicant has hereinabove cancelled claims 1-20 without prejudice and have submitted new claims 21-46. Applicant respectfully traverses the Examiner's rejection and requests that the Examiner hold the aforementioned rejection in abeyance until such time as prosecution on the merits is complete.

35 U.S.C. §102(b)

Applicant has cancelled claims 1-20 hereinabove without prejudice and submitted new claims 21-46. The Examiner has rejected claims 1-20 under 35 U.S.C. §102(b) as anticipated by U.S. Patent No. 5,102,662. ("Gallagher")

Applicant respectfully traverses the Examiner's rejection under 35 U.S.C. §102(b). New claim 21 recites:

> An insect repellant composition comprising a high vapor pressure compound and a low vapor pressure insect repellant, wherein the low vapor pressure insect repellant is soluble in the high vapor pressure compound.

New claim 33 recites:

> An insect repellant composition comprising a high vapor pressure compound, a low vapor pressure insect repellant, and an absorption

substrate carrier, wherein the low vapor pressure insect repellant is soluble in the high vapor pressure compound.

Applicant respectfully states that Gallagher does not disclose, teach or suggest *inter alia* an insect repellant composition comprising a high vapor pressure compound and a low vapor pressure insect repellant, wherein the low vapor pressure insect repellant is soluble in the high vapor pressure compound. Furthermore, Gallagher does not disclose, teach or suggest *inter alia* an insect repellant composition comprising a high vapor pressure compound, a low vapor pressure insect repellant, and an absorption substrate carrier, wherein the low vapor pressure insect repellant is soluble in the high vapor pressure compound. Gallagher merely describes DEET used as a contact repellant.

Applicant respectfully points out that prior to Applicant's invention DEET has been known and used only as a contact repellant and not an area repellant or combination contact/area repellant. See *Mosquito Magnets* in Science News Online by Corinna Wu attached as Exhibit A. In that article the author states that "DEET is a contact repellant, so the mosquito has to land on the chemical for it to work. A repellant that acts while in the air would be desirable but is a lot harder to come up with." See *Mosquito Magnets* at 5. Embodiments of the present invention provide the benefits of both a contact repellant and an area repellant, since the low vapor pressure insect repellant dissolves in the high vapor pressure compound (*e.g.*, a fragrance). At least a portion of this combination may be carried into the surrounding areas, while another portion may remain as a contact repellant on, for example, a substrate. Accordingly, embodiments of Applicant's invention solve the precise problem identified in the Science News Online publication.

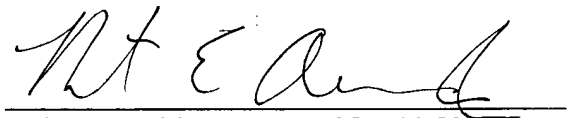
In view of the above, Applicant respectfully maintains that claims 21 and 33 are not anticipated by Gallagher. Likewise, Applicant maintains that those claims dependent on claims 21 and 33, namely claims 22-32 and 34-46, also are not anticipated by Gallagher.

Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection under 35 U.S.C. §102(b).

No fees, other than the fees for an application with excess claims and the fee for a one-month extension of time, are believed to be required in connection with the filing of this Amendment. However, if any additional fee is required, please charge our Deposit Account No. 50-0540.

Respectfully submitted,

Dated: October 31, 2003



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Home page.

Search

Archives

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 Tilt-A-Whirl Chaos (I)
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- **Science Safari**
 Engineering Top 20
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 70 Years Ago in *Science News*

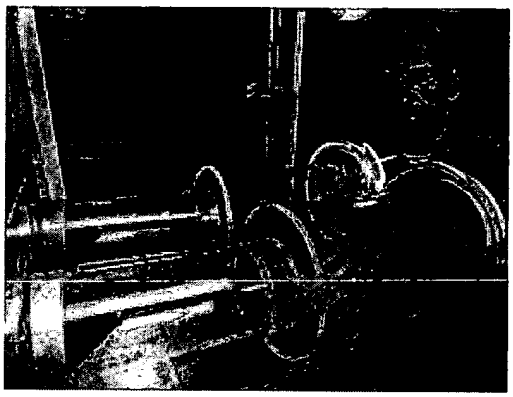
Mosquito Magnets

Your skin chemicals lure blood-sucking insects to their next meal

Corinna Wu

Ah, summer nights! The heat and humidity mingle with the sweet scent of citronella candles, the blue glow of a bug zapper, and the sticky feel of mosquito repellent. Some unfortunate people need this entire antimosquito arsenal to avoid getting eaten alive, while others hardly attract the pesky creatures at all.

Scientists have known for decades that mosquitoes are drawn to carbon dioxide, exhaled in abundance by the animals that hungry mosquitoes favor. Carbon dioxide doesn't tell the whole story, though.



ARS entomologist Donald R. Barnard places his hand inside the port of an olfactometer, a setup used to test mosquito attractants. Chemicals emanating from his skin lure mosquitoes toward a screen protecting his hand. Peggy Greb/ARS

Mosquitoes, after all, tend to bite people on the arms and legs. "I can't imagine that much carbon dioxide coming off of someone's hand," says Ulrich R. Bernier, a chemist at the U.S. Department of Agriculture's Agricultural Research Service (ARS) in Gainesville, Fla. "And how often do mosquitoes fly into your mouth or nose?"

Carbon dioxide is clearly important, but the skin must give off other attractants, too, he reasons.

In 1968, ARS scientists discovered that lactic acid, which emanates from human skin, acts as an attractant. Now, Bernier and his colleagues are trying to find other compounds emitted by skin that lure mosquitoes to their meals of blood. Using sensitive analytical techniques, they have catalogued more than 340 candidates. In the lab, the scientists are now offering some of these substances to mosquitoes to gauge their

attractiveness.

The researchers' ultimate goal is to develop better bug repellents and traps, a goal made more urgent by the threat of mosquito-borne diseases, says Bernier. Other scientists might also find biochemical, medical, or forensic applications for the results, he says.

Testing attractants

In the past, testing mosquito attractants required a brave volunteer to place an arm into a cage full of the insects. Luckily, the ARS group learned about 20 years ago that when a person handles glass, a residue that includes mosquito attractants is transferred from the skin. So instead of a live arm, researchers can often substitute a glass petri dish, which will attract mosquitoes for up to 6 hours.

Bernier wanted to find out what's in that mosquito-tempting residue. To collect samples, he and his colleagues asked volunteers to rub a handful of small glass beads between their palms for several minutes. The researchers then loaded the beads into a gas chromatograph-mass spectrometer, a device that separates mixtures of chemicals and identifies each component.



A female yellow fever mosquito probes a piece of Limburger cheese, one of few known mosquito attractants.
Peggy Greb/ARS

The glass beads enable researchers to trap oily skin emanations without capturing a lot of perspiration that would overwhelm the gas chromatograph with water. The technique also minimizes the collection of squalene, a precursor to cholesterol that's abundant on skin. In this way, the method unmasks the scarcer chemicals, Bernier says. He and his colleagues Matthew M. Booth and Richard A. Yost of the University of Florida in Gainesville described the technique in the Jan. 1, 1999 *Analytical Chemistry*.

The glass-bead sampling, however, doesn't capture the skin's most volatile components, which simply evaporate. Moreover, many water-soluble molecules don't show up in the samples because little water is collected. To get around these problems, the researchers would also wrap each volunteer's arm in a plastic bag and then drew out the humid air for a complementary analysis.

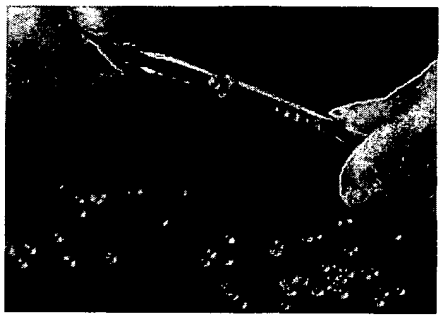
With these methods, Bernier and his colleagues have doubled the number of chemicals known to emanate from human skin. One day, more sensitive detection methods could further lengthen the list, he says.

"There could be well over 1,000 trace chemicals that I can't detect," he speculates. He and his colleagues present their latest findings in the Feb. 15 *Analytical Chemistry*.

Chemical reactions

To gauge the mosquitoes' reaction to chemicals, the researchers use a simple setup that they call an olfactometer. It consists of three clear plastic cages stacked on top of one another, each connected to a pair of cylindrical ports. Screens divide the attractants from the insects. Inside each cage, 75 female mosquitoes wait to be enticed. Only females bite; they need blood to nourish their developing eggs.

During a test, the researchers place potential attractants in the ports and blow gentle streams of air into the cage. If the mosquitoes like what they smell, they fly upwind toward the attractant. The researchers then tally the number of insects in the port.



Glass beads pick up scents after being handled. The beads will be loaded into an instrument that separates and identifies compounds in the residue. Peggy Greb/ARS

To account for differences in mosquito mood, the Gainesville group tests the three cages of mosquitoes in a random order at three different times of the day. "On some days, the mosquitoes don't respond as well. Other days, they're very active," says Bernier. The researchers run many trials and analyze their results statistically.

Mosquitoes show the best response to combinations of compounds, and it's time-consuming to find the right mix of attractants. Bernier winnowed attractants from the list of 346 chemicals by methodically mixing the skin's most abundant compounds with lactic acid. He has found that a blend of three particular chemicals attracts at least 90 percent of the mosquitoes in the olfactometer every time. Bernier's own arm and hand usually draw about 70 percent.

"It's a delicate situation," Bernier says. If the proportions aren't right, the mix of compounds leaves the mosquitoes unimpressed. Also, some compounds mask the real attractants, rendering them useless. "If you mix 30 compounds together, the mosquitoes don't even know [the attractant] is there," Bernier says.

The group has filed a patent on the blend of three compounds, which Bernier declines to identify.

In addition to pure chemicals, Bernier and his colleagues ran tests with skin residue from four people, all USDA employees, who varied in their mosquito appeal. The scent on glass beads handled by the tastiest

individual lured 77 percent, while the most repellent person drew only 23 percent of the mosquitoes.

Similarly, chemical analysis shows differences between the people.

Some smells that repel people lure mosquitoes, it turns out. Several years ago, Willem Takken and his colleagues at Wageningen Agricultural University in the Netherlands found that a species called *Anopheles gambiae* loves both stinky feet and Limburger cheese. A bacterium used to make Limburger is also found on the human foot, accounting for the similarity in odor, Takken says. The microorganism probably produces acids that draw the mosquitoes near.

ARS entomologist Daniel L. Kline tested the attractiveness of his own feet to mosquitoes in the lab and outdoors. He wore the same pair of socks 12 hours each day for 3 days. Alone, the socks did not draw many mosquitoes. When combined with carbon dioxide, however, the socks lured many mosquitoes, including disease-carrying species.

The researchers have never observed an attractant to draw 100 percent of the mosquitoes in the olfactometer, Bernier says, although recently one blend came close. The last mosquito began flying toward the attractant but made a U-turn at the last second and flew back into the cage.

Better mosquito traps

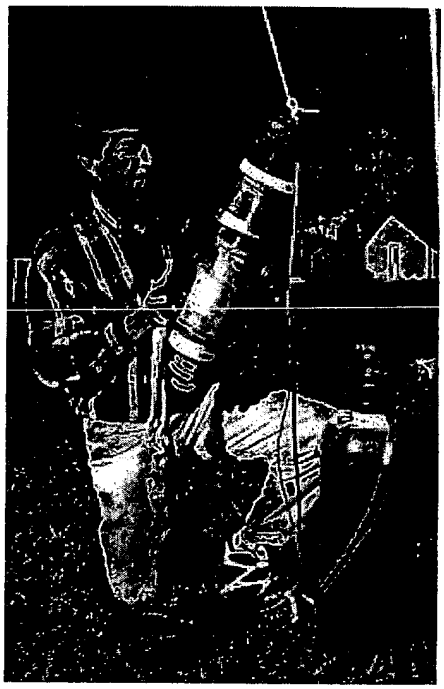
A combination of compounds that can beat out the scent of a person could lead to better mosquito traps. In many parts of the world, mosquitoes transmit diseases such as malaria, West Nile fever (SN: 12/11/99, p. 378: http://www.sciencenews.org/sn_arc99/12_11_99/bob1.htm), and encephalitis.

The Gainesville group always works with *Aedes aegypti*, also known as the yellow fever mosquito, because it's an easy species to study. Bernier says, "They'll bite anytime."

Other species, such as the malaria-carrying *Anopheles quadrimaculatus*, turn up their noses—or proboscises, rather—at human scents in the lab.

It's still too early to know whether the findings will yield useful products. To lure mosquitoes to a trap, an attractant must offer more appeal than the people nearby, which is a tall order to fill. "It's

very hard to approximate the human body. We don't know enough yet," says Bernier.



ARS entomologist Daniel L. Kline inspects mosquitoes caught in a collection device. A worn sock and carbon dioxide served as bait. (Peggy Greb/ARS).

Although by themselves some of the blends attract more mosquitoes than Bernier's arm, if both an arm and a blend are presented at the same time, the arm wins hands down.

However, if the researchers add a masking compound to the port with the human hand, the mosquitoes fly over to the port with the chemical blend. Perhaps a masking chemical could make a person less appealing than a nearby attractant baited trap.

It will be difficult to find something to compete with the commercial repellent DEET, or *N,N*-diethyl-3-methylbenzamide (SN: 4/27/96, p. 270: http://www.sciencenews.org/sn_arch/4_27_96/bob2.htm). "There's a push for natural repellents, but nothing compares to DEET in duration and effectiveness right now," Bernier says. "We're trying."

DEET is a contact repellent, so the mosquito has to land on the chemical for it to work. A repellent that acts while in the air would be desirable but is "a lot harder to come up with," says Bernier.

Another problem with DEET is that it doesn't work well against all mosquito species, Bernier notes. And although DEET is considered safe, some concerns linger about its effects on people (SN: 11/30/96, p. 347). Repellents based on naturally occurring compounds could minimize those fears, Bernier says.

Futuristic applications

The researchers' results have suggested some futuristic applications. The catalog of skin emanations could provide new ways to analyze criminal evidence, for instance. Chemicals in people's fingerprints seem to vary. Identifying prints' components could contribute information about the people involved in a crime (see "Chemicals in fingerprints could help solve crimes," below).

In a medical setting, the skin chemicals might give useful clues about health problems and drug use.

Bernier, however, focuses on insect control. One day, he says, people

may be able to take a pill that acts as a "systemic repellent" by altering the compounds given off by skin. The pill could cut back the production of attractive compounds or step up the production of ones that mask an enticing scent.

People then could stroll around with their own antimosquito force fields. They could turn off the bug zapper, snuff out the citronella candles, throw out the sprays, and just enjoy the summer nights.

Chemicals in fingerprints could help solve crimes

The chemicals released by skin not only lead mosquitoes to their prey, but they might also lead police officers to criminals.

Chemist Michelle V. Buchanan and her colleagues at Oak Ridge (Tenn.) National Laboratory (ORNL) are identifying the chemical compounds in fingerprints. The researchers are looking for patterns in the chemical composition of sample prints from 300 volunteers. This type of information could help law enforcement officers assemble profiles of criminal suspects.

Buchanan's foray into fingerprints began with an observation made in 1993 by Art Bohanan, a detective with the Knoxville Police Department. While gathering evidence related to the abduction and murder of a 3-year-old girl, police could find none of the girl's fingerprints in the primary suspect's car. The suspect's prints, however, were all over the vehicle. A similar situation had occurred during an earlier child-abduction case.

Bohanan suspected that the kids' fingerprints had somehow disappeared, so he decided to conduct an experiment. He asked several children and adults to handle clean glass and plastic soda bottles. He kept half of the bottles in his cool basement and the other half in the back seat of his car. Each day for a month, he removed bottles from both places and dusted for fingerprints. In the warm car, the children's prints began fading right away and often disappeared within 24 hours. In the basement, all the prints remained longer. Under both sets of conditions, the adult fingerprints lasted, on average, four times as long as the children's prints.

Bohanan asked ORNL scientists for help in solving this mystery. Buchanan and her colleagues had 50 volunteers shake open-ended vials of rubbing alcohol between thumb and forefinger, thus collecting samples of compounds from their fingertips. Half of the volunteers were between 4 and 12 years old, and the other half, between 17 and 46 years old. The scientists then used gas chromatography and mass spectrometry to separate and identify the chemicals dissolved in the alcohol.

They found dramatic differences. The adult fingers gave off more oily, long-lasting compounds such as fatty acid esters than the children's did. The kids' samples contained more cholesterol and volatile chemicals, such as free fatty acids, than the adults' did. Most of the components released by kid's fingers simply evaporate, Buchanan realized.

Subsequent tests also turned up estrogen and testosterone. One of the adult samples showed nicotine.

An upcoming ORNL study will collect skin compounds with a technique similar to the one used by the Agricultural Research Service in Gainesville, Fla. Volunteers will rub between their hands some glass beads, the kind sold in hobby shops for flower vases. Buchanan and her group will then analyze the composition of the residue left on the beads.

If patterns arise, police and forensic scientists might be able to assemble rough profiles of suspects based on the chemical composition of fingerprints. Analyzing skin chemicals might also be a noninvasive way to test for medical disorders or drug use, says Buchanan (SN: 2/7/98, p. 88).

"Your skin is such a huge organ. With all the pores that you have, it's a major way for your body to get rid of things," she notes.

The work could also lead to more effective dyes for fingerprint dusting. At the very least, the findings have taught Bohanan and his fellow officers to look for kids' prints as soon as possible. That way, missing fingerprints won't worsen the pain of parents who are missing a child.

Letters:

In doing your usual excellent job of presenting information in interesting and lighthearted ways, you implied that bug zappers control mosquitoes. In fact, bug zappers don't attract mosquitoes and therefore kill very few of them. They do kill large numbers of harmless and even beneficial insects, including pollinators and insects such as the crane fly, which would be out there eating mosquitoes if they weren't zapped.

Merle Hetley
Bangor, Maine

I found the article interesting. Perhaps the researchers could confirm or refute the old saying "swat a mosquito (or blackfly), and 100 will come to the funeral." My father used to say he was quoting his father. That would put the saying back to the mid-1800s at least.

Alden Gray

Ashfield, Mass.

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From *Science News*, Vol. 157, No. 17, April 22, 2000, p. 268.

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